THE BIOLOGY AND CONTROL OF NEPTICULA SLINGERLANDELLA KEARFOTT ON TART CHERRIES IN MICHIGAN

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

RUEDIGER CARL HILLMANN

1967

ABSTRACT

THE BIOLOGY AND CONTROL OF

NEPTICULA SLINGERLANDELLA KEARFOTT

ON TART CHERRIES IN MICHIGAN

by Ruediger Carl Hillmann

Body of Abstract

A biological study of *Nepticula slingerlandella* Kearfott, a serious leaf miner of Montmorency tart cherry, *Prunus cerasus* L., was conducted in Michigan during 1966 and 1967.

Adult moths emerged during late-bloom and petal-fall of the host. The adult population contained primarily males during the first week of emergence; however, at the time of peak adult activity the sex ratio was 1:1. Adult longevity averaged 11 days. The mean fecundity was 17.9 eggs per female. Egg incubation averaged 16 and 19 days under field conditions and 11 days in the laboratory at 80 F.

Four larval instars were determined by head capsule measurement and supplemental larval development observations. Indications of larval and pupal parasitism were noted.

Additional host plants confirmed in Michigan were plum, *Prunus domestica* L., sweet cherry, *Prunus avium* L., pin, red, or fire cherry, *Prunus pennsylvanica* L., and peach, *Prunus persicae* L. Mining could not be induced on wild black cherry, *Prunus serotina* Ehrh.

Ruediger Carl Hillman

Chemical control studies were conducted in 1964, 1966 and 1967. Several insecticides gave excellent control. The application of pesticides at petal-fall followed by a first-cover spray 10 days later for control of the adult was the preferred treatment. First-and second-cover sprays against eggs and larvae also gave good control.

THE BIOLOGY AND CONTROL OF

NEPTICULA SLINGERLANDELLA KEARFOTT

ON TART CHERRIES IN MICHIGAN

by

Ruediger Carl Hillmann

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1967

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to his major professor, Dr. A. J. Howitt. His encouragement, patience and financial support added incentive toward completion of this research program.

Special thanks go to Dr. Paul H. Wooley, who became the writer's major professor in Dr. Howitt's absence, for guidance in the final completion of this thesis.

The writer's sincere gratitude is extended to Drs. Gordon E. Guyer, James E. Bath, and John H. Hart for serving on his guidance committee.

The writer is grateful to colleagues Paul E. Boldt and Richard F. Gertz for their assistance in the collection of data. Special thanks, also, to technicians Al Pshea and Ian Richards for their help in carrying out the chemical control experiments.

A special award of appreciation (Ph.T.) to my wife, Julia, whose love, patience and humor were so important on innumerable occasions.

A final thanks to all those persons who have assisted the writer in any way during this endeavor.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

INTRODUCTION

Infestations of a leaf miner, tentatively identified as *Nepticula sp.*, caused severe defoliation in a number of Montmorency tart cherry orchards, *Prunus cerasus* L. in southwestern Michigan in 1962.

In 1964, 1966 and 1967 chemical control experiments were conducted in a one-half acre block of infested tart cherry trees located near Lawrence, Michigan. An intensive study to determine the biology of this pest was conducted in 1966 and 1967 at Lawrence, Shelby and Oshtemo, Michigan.

The research objectives were:

1. Identify the insect.
2. Determine the insect's life history, host range, and distribution in Michigan.
3. Investigate the extent and importance of natural controls.
4. Determine effective chemical controls.

IDENTIFICATION

Six adults and one wing-slide were sent to Dr. Annette Braun, Professor Emerita Ohio State University, an international authority on the family Nepticulidae for identification. Dr. Braun identified the specimens as *Nepticula slingerlandella* Kearfott.

Fifty adults and 12 larvae collected from infested orchards at Shelby and Lawrence, Michigan were sent to Dr. D. R. Davis, who is affiliated with the United States National Museum. Dr. Davis identified the material as *Nepticula slingerlandella* Kft.

Although this insect has no accepted common name, it is referred to as the "plum leaf miner" by Crosby (1911) and Slingerland (1909) in New York. It is commonly called the "cherry leaf miner" in Michigan.

LITERATURE REVIEW

Nepticula slingerlandella was found by Braun (1917) in Ohio and by Crosby (1911) and Slingerland (1909) near Rochester, New York.

The adult was described by Kearfott (1908) as follows: "*Nepticula slingerlandella*, new species. Expanse 3.5 to 5 mm. Head and frontal tuft orange, antenna eye-caps and side tufts white; antenna whitish gray; thorax bronzy black; abdomen light gray; legs yellowish white, posterior tarsi finely ringed with black. *Forewing*-Bronzy black with a shiny fascia at outer third. Cilia and hind wings light gray."

Braun (1917) stated that the frontal tuft becomes "paler behind where it merges into the pale ocherous or whitish collar." In addition she noted the somewhat irrorated texture of the forewing beyond the fascia.

Crosby (1911) states, "These moths emerge from cocoons at or near the surface of the ground during the daytime in the latter part of May and early June. During the day they remain quietly on the bark of the trunk and larger branches, none being found on the leaves. Several hundred moths are often found on a single tree. They gradually decrease in numbers, and about the middle of June, they disappear."

Crosby (1911) reports the following information about the egg: "The eggs are attached to the under surface of the leaf, usually at the forks of the more prominent veins. The egg is about 0.3 mm long by 0.2 mm wide, oval in outline, flattened where attached to the leaf and dome shaped in profile. The green of the leaf shows through the transparent egg shell, making it a difficult object to find. The eggs are most easily located by holding the leaf at an angle in the sun so that the light will strike it obliquely, when they will be seen as minute glistening dots." He also states that the time required for hatching "cannot be far from two weeks."

Crosby (1911) reports that "in hatching, the larva eats its way out of the eggshell on the underside next to the leaf, and enters the leaf directly without coming out on the surface." He continues, "When full grown, the larvae are about one-sixth inch in length, greenish white in color with the head light brown. The contents of the alimentary canal show through the semi-transparent body as a greenish or brownish stripe."

Needham (1928) describes the larvae of Nepticulidae as follows: "The larvae are slightly flattened with the head deeply retracted into the thorax. Jointed thoracic legs and crochets are wanting and locomotor appendages are represented only by roughened protuberances. Such projections are to be found in pairs on the second and third thoracic segments and on the second to seventh abdominal ones. Some species are said to have one or two additional pairs."

Crosby (1911) reports only one generation in New York State. Braun (1917) reports that a second brood of larvae may be collected in September "farther south" (than New York).

Braun (1917) reports that the larvae of *Nepticula* have four larval instars. Lindquist (1962) determined four larval instars in *Nepticula lindquisti* Freeman, a leaf miner of birch.

Braun (1917) states that a change in the character of the mines of *Nepticula* usually indicates the beginning of a new instar. "The mine formed during the first instar is very short, rarely exceeding a few millimeters in length. The large conspicuous part is made during the last larval instar."

Crosby (1911) describes the mine as a narrow linear burrow, often tortuous in its early course, that enlarges "into an irregular ovate blotch about one half inch in length. In the linear part of the mine the excretement is left as a blackish streak extending along the center of the burrow; in the blotch mine it forms a broad, irregular band along the center, but does not extend to the tip."

Braun (1917) reports that larvae of *Nepticula* usually mine "just beneath the upper epidermis, consuming the palisade layer of cells and in later stages, some of the spongy parenchyma cells."

Crosby (1911) writes, "when full grown the larva leaves the mine through a cut in the upper surface of the leaf, falls to the ground and there constructs a small flattened brownish cocoon . . . broadly oval and moderately arched, it is about 2 1/2 mm long by 1 1/2 to 2 mm wide, and is usually wider at one end. It is surrounded by a thin flange formed by the closely united edges of the two valves of which the cocoon is composed." He continues, "the pupae is about 2 mm in length, ovate pointed behind and somewhat flattened. The ventral surface is brownish yellow, the dorsum greenish. The eyes are dark colored and the orange tuft on the head of the moth shows through the pupal skin. On the dorsum of the abdomen are six transverse interrupted rows of short brownish spines. On each side of the dorsum is a longitudinal row of wart-like protruberances each bearing a colorless spine. The anterior spines are very short and they gradually increase in length towards the tip of the body. When about to transform to the adult, the pupa works itself partly out of the end of the cocoon, possibly by the aid of these spines."

Incidence of pupal parasitism was reported by Crosby (1911). The parasite was a chalcis-fly, Derostenus salutaris Crosby. The larva of the parasite emerged through a small round hole in the side of the parasitized pupa's cocoon. "In the fall of 1911 the parasites had increased in numbers so that nearly one half of the cocoons were infested."

Slingerland (1909) and Crosby (1911) state that the insect attacked both plum and prune in New York. Both Crosby (1911) and Braun (personal letter) found mines on apple closely resembling N. slingerlandella mines on tart cherry. Braun (1917) reports that the insect mined wild plum, Prunus americana, and occasionally sweet cherry, Prunus avium L.

A number of *Nepticula* species closely resembly *N. slingerlandella*, either in the external appearance of the adult or in the hosts attacked by the larva. Braun (1917) in her dissertation on the family Nepticulidae describes the following species: *N. bifasciella*, Clemons, which Dr. Braun considers to be identical to both *N.*? *prunifoliella* and *N. serotinaella*, mines wild black *Prunus serotina*, and occasionally wild plum, *P. americana*. Another species, *N. rosaefoliella* Clemons, is a leaf miner of various species of rose.

Crosby (1911) describes a European species, *N. plagicolella* Stainton, which is closely related to *N. slingerlandella*, that feeds on cultivated plum. Lindquist (1962) published a life history of a birch leaf miner, *N. lindquisti* Freeman, the adult of which resembles *N. slingerlandella* very closely.

MATERIAL AND METHODS

Areas in four commercial tart cherry orchards were secured for this study. A 1/2-acre block near Lawrence (Van Buren County) was employed for intensive biological studies in 1966 and 1967. Preliminary chemical control studies had been conducted in this orchard in 1964. An 8-tree block near Shelby (Oceana County) was used in 1966, but studies were discontinued in 1967 because small numbers of *N. slingerlandella* were found at that time. Another Shelby block, consisting of 35 trees, was used in 1967. An Oshtemo block (Kalamazoo County), consisting of 30 trees, contained the lightest infestation of *N. slingerlandella* and was used in 1967 only.

Laboratory experiments conducted at East Lansing were concerned with adult emergence, behavior, oviposition, egg incubation, larval instar determination and pupal diapause.

EXPERIMENTS, OBSERVATIONS, RESULTS AND DISCUSSION

Laboratory Emergence Studies

To predict adult emergence in the orchard and to secure adults for positive identification, *N. slingerlandella* adults (Figs 1-2) were induced to emerge from pupae contained in orchard litter. Litter gathered at various times was incubated in rectangular 12 x 14 x 16-inch wooden cages covered with 32-mesh saran screening. The cages were placed in a rearing room maintained at 70 F. with 55 to 60% relative humidity. A 24-hour photoperiod was supplied by two 80-watt fluorescent tubes.

Two adults emerged on April 12, 1966 from litter that had been incubated continuously since March 26. By April 20, 47 additional adults had emerged. Adult studies in the laboratory were continued through June 10, 1966 with the use of fresh litter. This procedure predicted field emergence of adults very closely. Seven adults emerged in the laboratory on May 23, 1966 from Lawrence litter incubated on May 21. Emergence in the Lawrence orchard was confirmed by a backlight trap catch of 23 adults on May 25.

On April 19, 1967, infested litter from Lawrence was incubated in a saran-screened cage and on April 29 three adults emerged. These data were compared with 1966 data from a comparable calendar period. An algebraic proportion computation indicated that emergence would occur at the Lawrence orchard

Fig 1.-Nepticula slingerlandella moth on a tart cherry leaf.

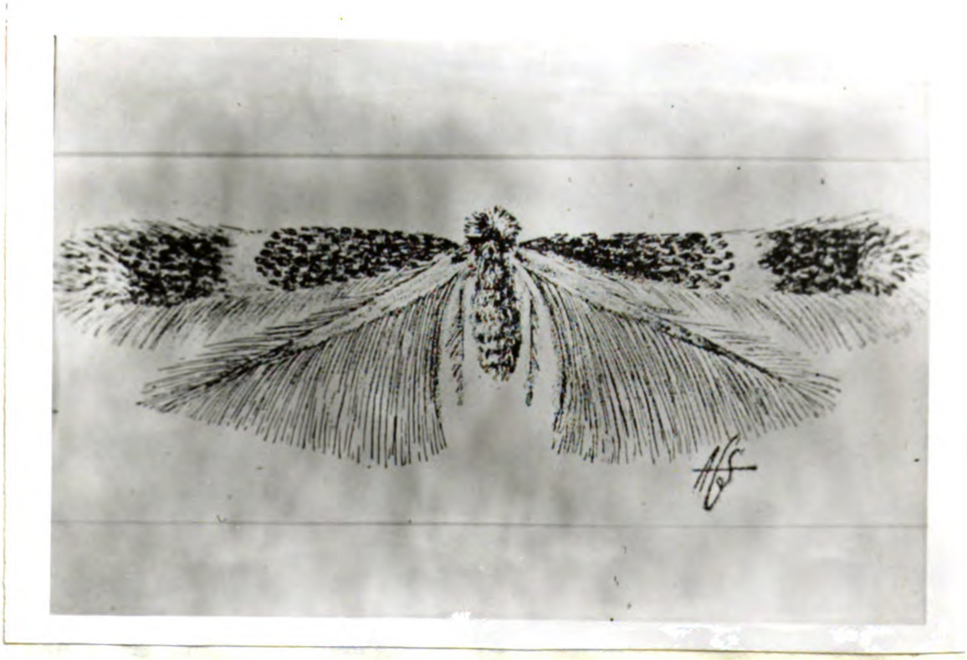

Fig 2.-Illustration of a Nepticula slingerlandella moth. (From Crosby, C. R. 1911. Cornell Univ. bulletin 308)

on May 11. Emergence at Lawrence was confirmed with a blacklight catch of five males on May 14. Adult emergence in the field, therefore, may be predicted with reasonable accuracy from emergence induced in the laboratory.

To ascertain if light affected adult emergence, litter was placed in six 1-quart, cylindrical cardboard containers with tight-fitting lids and incubated in the rearing room. Containers were checked daily for adult emergence. Emergence within these dark containers closely paralleled the emergence that took place in the lighted saran-screened cage.

Crosby (1911) stated that the moths emerge during the daytime. While this is true, the fact that emergence took place in dark containers indicates that the moths may also emerge at night.

Field emergence data for 1966 indicated a 3-week lag in adult emergence between Lawrence and Shelby. To determine if such a lag would occur under laboratory conditions, litter gathered on April 18, 1967 at Shelby and on April 19, 1967 at Lawrence was placed in adjacent saran-screened cages in the rearing room on April 20. Emergence from the Lawrence litter occurred on April 29; emergence from the Shelby litter did not occur until 16 days later. Pupation at Lawrence occurred 2 to 3 weeks earlier than at Shelby in 1966. This may have influenced the time of adult emergence in 1967.

Laboratory Behavioral Studies

A seedling Montmorency tart cherry tree was placed in the greenhouse in April 1966 under conditions of natural photoperiod to study the behavior of the adults and determine whether they required nourishment. In addition, the tree provided an opportunity to confirm the formation and development of leaf mines. Two branches, were each enclosed in a separate, 6-inch cylindrical saran screen (32 mesh) cage 18 inches high, fitted over a clay pot containing sterilized greenhouse soil. The pots were placed in individual saucers filled with water. One cage was equipped with an internal sucrose dispenser, consisting of a small wad of cotton that was saturated daily with sucrose solution. In addition, this cage was equipped with a continuous water dispensing device. One end of a 3-inch wide cotton strip 1-foot long was placed in a container of water. The remaining 6 inches were taped to the exterior of the cage and water was allowed to move upward by capillary action. The other cage lacked these water and nourishment devices. Adults that had emerged in the rearing room were introduced into both cages. Adults in the cage without added nourishment died within 2 to 3 days, probably from desiccation rather than from lack of nutrients since the adults normally feed on leaf glands. Several adults in the other cage were still active after 14 days. Leaf mining occurred in both trials indicating that oviposition may occur within 2 to 3 days after emergence.

Adult Emergence in the Orchard

A 15-watt "Spin-sect" blacklight insect trap (Fig 3), manufactured by Ampsco Corporation, Columbus, Ohio, was erected in each of the test blocks to determine adult emergence in the orchard and to measure adult activity. Blacklight traps were placed in Lawrence in 1966 and 1967 and in the Shelby and Oshtemo blocks in 1967. Each light was firmly fastened to a cherry tree approximately 4 feet above ground level. In 1966 electrical power was supplied by a 12-volt battery, which was charged daily, whereas in 1967 the lights were connected directly to 110-volt electrical outlets. Insects were collected daily from the traps throughout the season.

To determine the time of adult emergence in 1966 in the Shelby area two flat black planks 2 inches thick and 12 inches sq were coated with "tangle-foot" and suspended 3 feet above ground level in two cherry trees in the Shelby orchard. Insects were identified and removed from the boards twice weekly.

The malaise trap (Fig 4) was used during the 1966 season at Lawrence. The trap was approximately 7 ft high and 4 ft sq. The collection device consisted of a screen and plastic funnel at the top of the trap connected to a collection jar containing alcohol.

In 1966 the insects were attracted by the black portion of the trap; thus, in 1967 a trap consisting entirely of black

Fig 3.-A "Spin-sect" blacklight insect trap used for adult emergence studies.

Fig 4.-The malaise trap used in 1966. The trap used in 1967 was completely black with an opaque collecting device in place of the screen and plastic one shown above.

material and utilizing an opaque collection device connected to a 1-quart jar containing alcohol was used. The interior of the trap became extremely hot during periods of sunlight. To correct this problem a large white sheet was placed over the upper half of the trap. This trap did not capture any N. slingerlandella adults in 8 weeks of operation.

The emergence curves developed for N. slingerlandella are presented in Fig 5, 6, 7 and 8. Daily catches are given in Tables 1, 2 and 3. Large daily blacklight samples were carefully divided into equal sub-samples, each of which contained at least 100 N. slingerlandella adults. The sub-samples were taken by evenly spreading the complete sample within a 4-in. square (16 sq. in.) drawn on a 12 x 16 in. sheet of bristle board. The 16 sq. in. area was sub-divided into four smaller squares of 4 sq. in. each (2 x 2 in.). One of the latter was again subdivided into four 1-sq. in. units (1 x 1 in.). Therefore, a grid system was constructed whereby the complete sample could be divided into halves, quarters, eights, and sixteenths. Since it was decided that each sub-sample should contain at least 100 N. slingerlandella adults the area counted was governed by specimen density. Successively larger grids were counted until at least 100 individuals had been enumerated. The number and sex of adults in a sub-sample was multiplied by the inverse of the sub-sample fraction to arrive at a complete sample count, which is the number reported. For example, a 1/4 sub-sample that contained 100 adults would be reported as a

Table 1.-Comparison of blacklight and malaise traps in sampling adults of <u>Nepticula slingerlandella</u>. Lawrence, Michigan. 1966.

No. of Adults			No. of Adults		
Date	Blacklight	Malaise trap	Date	Blacklight	Malaise trap
May 24	0	0	June 11	72	5
25	23	3	12	44	2
26	9	2	13	16	0
27	22	5	14	2	trap removed
28	0	0	15	8	
29	-[a]	5	16	-[a]	
30	-[a]	8	17	4	
31	-[a]	9	18	0	
June 1	18	2	19	39	
2	82	10	20	10	
3	79	14	21	8	
4	25	2	22	0	
5	133	7	23	19	
6	304	14	24	7	
7	4	2	25	3	
8	40	3	26	0	
9	16	5	27	1	
10	0	10	28	0	

[a]no samples taken this date

Table 2.-Blacklight trap catches of Nepticula slingerlandella adults. Lawrence, Michigan. 1967.

	No. of Adults					No. of Adults			
Date	Total	♀	♂	Non-det.[a]	Date	Total	♀	♂	Non-det.[a]
May 13	0	0	0	0	May 29	139	18	109	12
14	5	0	4	1	30	424	44	360	20
15	1	0	0	1	31	356	72	284	0
16	7	0	5	2	June 1	1520	364	1088	88
17	52	0	48	4	2	784	144	626	24
18	13	1	12	0	3	2544	736	1520	288
19	3	0	3	0	4	220	56	144	20
20	21	0	20	1	5	132	42	80	10
21	14	1	13	0	6	326	144	118	64
22	32	1	31	0					
23	10	1	9	0	8	16	8	8	0
24	110	11	82	17	9	9	6	3	0
25	108	34	55	19	10	2	1	1	0
26	1057	159	807	91	11	1	1	0	0
27	153	40	137	16	12	0	0	0	0
28	66	5	57	4	13	0	0	0	0

[a]Non-det. = Sex could not be determined.

Table 3.-Blacklight trap catches of <u>Nepticula</u> <u>slingerlandella</u> adults. Shelby, Michigan. 1967.

Date	No. of Adults Total	♀	♂	Non-det.[a]	Date	No. of Adults Total	♀	♂	Non-det.[a]
June 1	0	0	0	0	June 21	146	14	126	6
2	2	0	2	0	22	103	4	92	7
3	0	0	0	0	23	736	208	440	88
4	2	0	2	0	24	77	29	42	6
5	14	0	14	0	25	108	22	80	6
6	3	0	3	0	26	84	16	66	2
7	0	0	0	0	27	164	58	100	6
8	3	1	2	0	28	120	42	63	15
9	19	1	15	3	29	226	50	152	24
10	8	2	5	1	30	274	50	254	20
11	33	0	31	2	July 1	70	16	44	10
12	5	0	4	1	2	17	7	9	1
13	76	7	54	15	3	26	1	24	1
14	53	8	31	14	4	63	22	41	0
15	178	16	149	13	5	11	8	3	0
16	500	28	344	128	6	56	18	38	0
17	336	12	288	36	7	44	9	35	0
18	22	4	14	4	8	16	6	10	0
19	202	14	164	24	9	7	4	3	0
20	541	32	481	28	10	0	0	0	0

[a]Non-det. = Sex could not be determined.

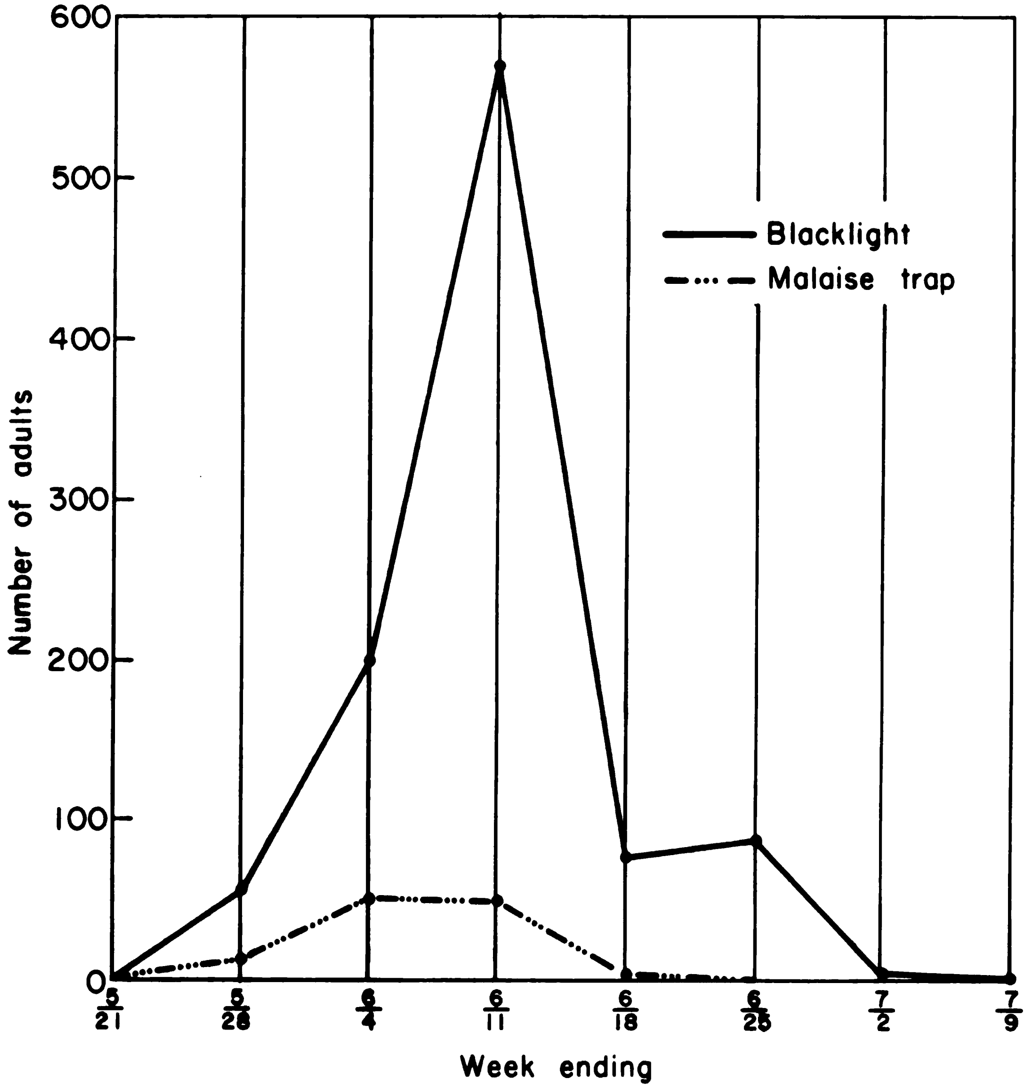

Fig 5.-Emergence curve of Nepticula slingerlandella as determined from blacklight and malaise trap captures of adult moths. Lawrence, Michigan. 1966.

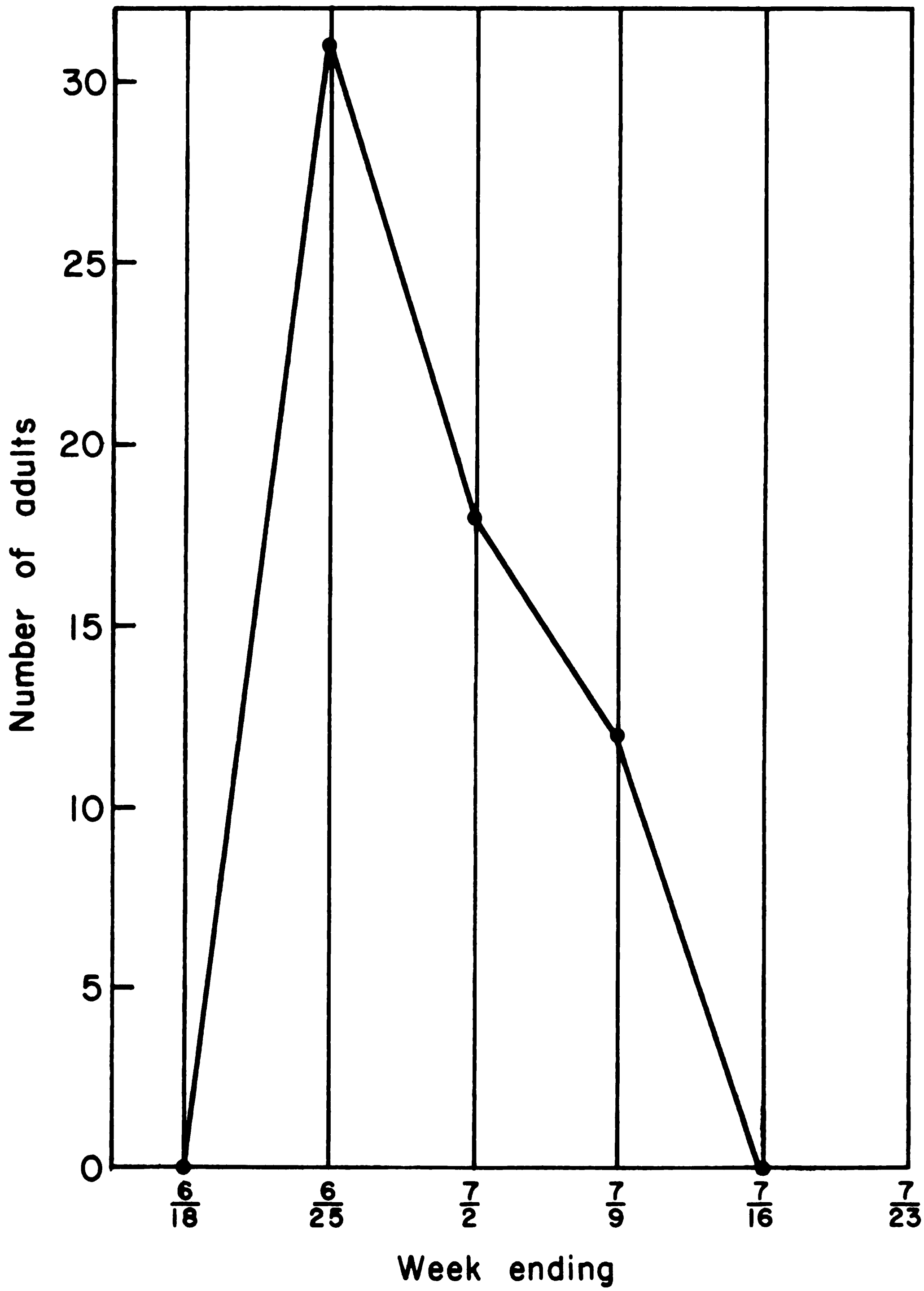

Fig 6.-Emergence curve of <u>Nepticula slingerlandella</u> as determined from two black sticky board captures of adult moths. Shelby, Michigan. 1966.

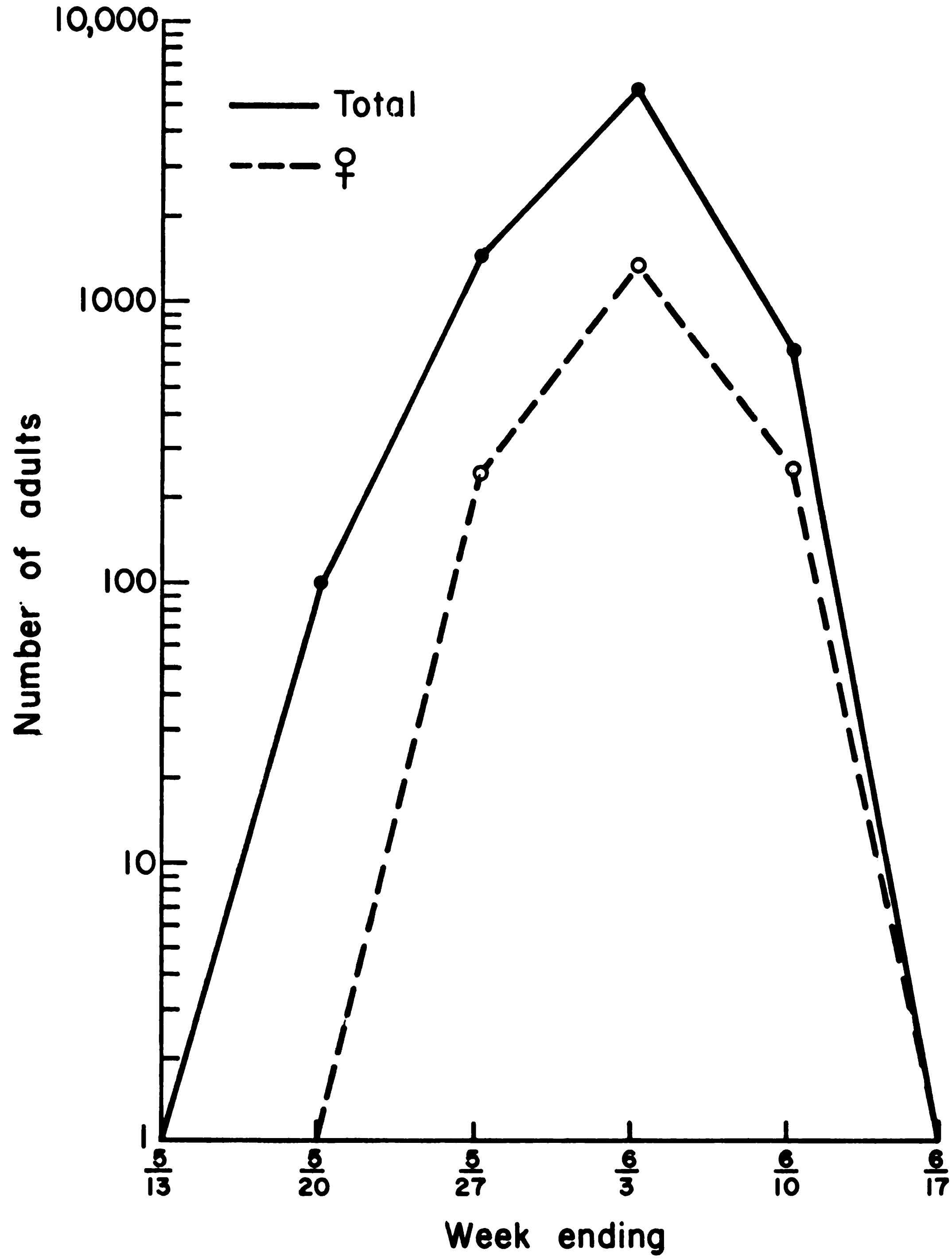

Fig 7.-Emergence curve of <u>Nepticula slingerlandella</u> as determined from blacklight trap captures of adult moths. Lawrence, Michigan. 1967.

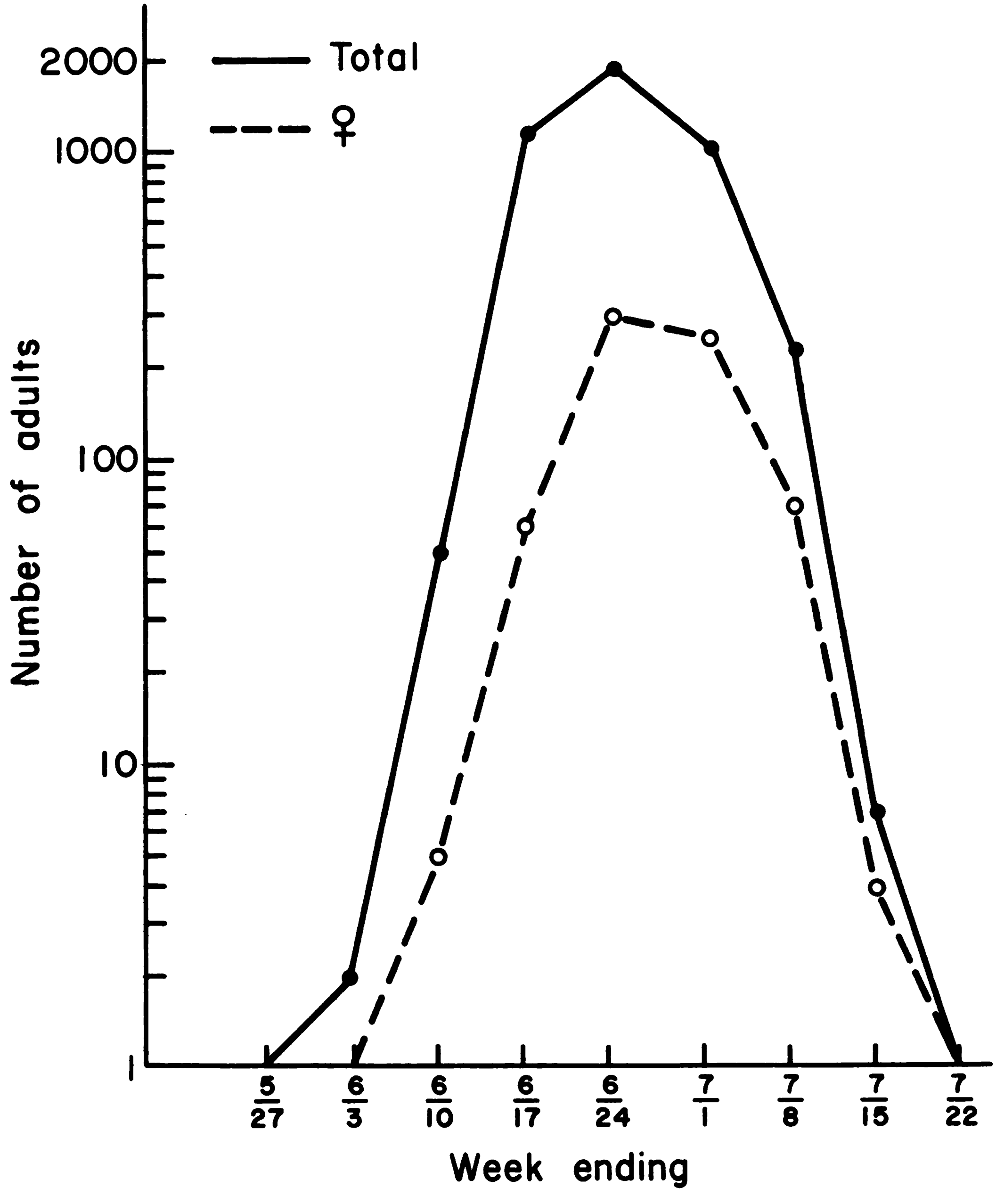

Fig 8.-Emergence curve of Nepticula slingerlandella as determined from blacklight trap captures of adult moths. Shelby, Michigan. 1967.

whole sample count of 400 adults for that date.

Although adults may be collected for a period of 6 to 8 weeks in orchards infested with N. slingerlandella, peak flight occurred within 2 to 3 weeks after initial emergence after which the numbers of adults declined rapidly. Females emerged 1 week later than the males, peaked simultaneously with the males and probably outnumbered the males in the last half of the adult activity cycle. The light trap samples contained a majority of males throughout the sampling period because of the male swarming behavior at daybreak. The preponderance of males early in the emergence cycle enhances the probability that copulation will occur soon after female emergence.

The lower peak observed at Lawrence in 1966 (Fig 5) is probably due to insufficient power being supplied by the battery. The Shelby population lags 2 to 3 weeks behind that at Lawrence. The graphs also indicate the annual environmental variation that affects the time of insect emergence. Emergence at both locations, in both years, occurred during late bloom and petal fall. While the calendar emergence dates vary considerably, there is agreement between emergence and phenological development of the tree.

A single generation per year is beneficial to this species, since the majority of any second brood would perish from starvation. The first brood causes such extensive defoliation that little foliage is left for any prospective second brood. In addition, the leaf glands, from which the adults derive much of their nourishment, dry up after the leaf matures (Reinke 1876 in Esau 1953). Thus, any second generation adults would be subjected to very adverse conditions.

To determine if other types of insect traps were effective in capturing *N. slingerlandella*, a dozen sticky boards of three different colors were tested at the Lawrence block. Single black, green and yellow sticky boards measuring 1 x 8 x 10 inches were suspended about 4 ft above ground level in each of four trees selected at random throughout the Lawrence orchard. They were positioned approximately equidistant from each other around the tree. The number of trapped *N. slingerlandella* adults was recorded weekly (Table 4).

Although there is considerable variation between replications, the green sticky boards trapped more insects than the other two colors combined. With a single exception, peak catches were recorded for all color trials by May 30.

It is likely that the boards are not sufficiently precise to warrant their use for experimental purposes. However, the green-colored boards are satisfactory as a base for the timing of chemical applications for control of *N. slingerlandella*. From a practical standpoint, the phenological development of the host is a sufficiently accurate guide upon which to base commercial control.

A study was initiated to determine the sexual pattern of adult emergence in the orchard. Four ground cages, one, 36 x 36 x 36 inches and the other three, 16 x 24 x 20 inches,

Table 4.-Comparison of three colored sticky boards as traps for N. slingerlandella adults. Lawrence, Michigan. 1967.

Tree	Color of Board	No. of N. slingerlandella recorded on specified date						Seasonal Total
		5/16	5/22	5/30	6/5	6/14	6/21	
A	Black	1	18	79	43	0	0	141
	Green	0	65	400	65	1	0	531
	Yellow	0	8	114	53	0	0	175
B	Black	0	0	30	14	1	0	45
	Green	0	2	250	37	0	0	289
	Yellow	0	1	45	15	2	0	63
C	Black	0	3	178	21	0	0	202
	Green	4	32	610	32	1	0	669
	Yellow	0	1	104	54	0	0	159
D	Black	0	0	43	35	4	0	82
	Green	0	1	31	17	8	0	57
	Yellow	0	1	47	147	17	0	212
Total	Black	1	21	330	113	5	0	470
	Green	4	100	1281	151	10	0	1746
	Yellow	0	11	310	269	19	0	609

were placed open end down at random under trees to trap adults as they emerged. The adults that emerged in these cages were sexed and recorded (Table 5). While males predominate during the early emergence cycle, females emerge in sufficient number to balance the sex ratio and predominate during the last half of the adult activity period.

Adults that had been periodically collected at random in the orchard were sexed to determine the changing seasonal sex ratio under orchard conditions at Lawrence (Table 6). While these data showed a trend similar to that shown by the ground cages, the sex-ratio changes appear to be a week behind the changes shown by the ground cages. The tree samples represented the total *N. slingerlandella* population present in the orchard, while the ground cage data represented only the emergence that had taken place within the sample interval.

Field-collected adults were sexed on consecutive days in similar areas in Oshtemo (Table 6). The data demonstrate the great variation in the sex ratio that may occur in a short interval of time. Field-collected adults were sexed on only 1 day in Shelby (Table 6) and as in Lawrence, males predominated during the first half of the emergence period.

The blacklight trap is the most effective method for sampling field activity of this insect. The ground traps sample the current adult emergence more effectively because samples are unaffected by previous emergence. The area sampled, however, is much more limited.

Table 5.-Emergence of N. slingerlandella as determined by four ground cages. Lawrence, Michigan. 1967.

Date	Total Adults Trapped	Male		Female	
		Number	%	Number	%
May 24	99	63	63.7	36	36.3
26	228	152	66.7	76	33.3
28	246	128	52.0	118	48.0
30	143	63	44.0	80	56.0
31[a]	33[a]	10	30.3	23	69.7
June 14	1	--	--	1	100.0

[a]From large ground trap only.

Table 6.-The percentage of females in samples of *N. slingerlandella* adults collected from tart cherry trees. Lawrence, Oshtemo and Shelby, Michigan. 1967.

Date	No. of Adults	% Females
Lawrence		
May 19	48	12.5
21	100	15.0
22	106	21.7
24	97	15.5
26	123	28.5
31	50	40.0
Oshtemo		
May 24	82	17.1
25	167	37.1
Shelby		
June 20	125	32.8

Orchard Observations of the Adult

To analyze the nocturnal behavior of N. slingerlandella, adult insects were collected hourly from the light trap at Lawrence from 8 PM through 6 AM on May 26, 1967. The temperature ranged from 61 to 68 F.

A relatively high number of females was caught between 8 and 10 PM (Table 9). These females became active soon after dusk, left their resting places at the base of the tree trunk, and began their nightly migration up the tree past the blacklight. The number trapped declined after 10 PM since the majority of adults were in the upper branches beyond the range of the blacklight.

The heavy concentration of males occurred between 4 AM and daybreak when the males became very active and formed a mating swarm. Many were attracted to the blacklight and consequently all seasonal blacklight samples, with the exception of a few collected at the end of the adult activity period, showed a preponderance of males.

It would have been desirable to operate the light trap for two additional hours. It is likely that the insect count would decline rapidly between 6 and 7 AM then drop to near 0, since many adults are mating and the remainder are resting quietly on the trunks of cherry trees.

Field sexing was based on abdominal shape; the females have a thick sausage-shaped abdomen, while the males have a much thinner funnel-shaped abdomen. Laboratory sexing may be

Table 7.-Hourly blacklight trap catches of N. slingerlandella adults. Lawrence, Michigan. May 26, 1967.

Hour	No. of adults caught			
	Total	♀	♂	Non-det.[a]
8-9 PM	91	33	48	10
9-10	61	32	25	4
10-11	18	7	10	1
11-12	43	9	24	10
12-1 AM	18	5	10	3
1-2	13	2	9	2
2-3	28	5	22	1
3-4	16	1	13	2
4-5	391	42	320	29
5-6	378	23	326	29
Total	1057	159	807	91

[a]Non-det. = Sex could not be determined.

done by noting the four valve clasper formed by a mesal horizontal and vertical cleft of the male's posterior; the female has no readily visible external genital organs.

Adult behavior patterns were observed on numerous occasions in the field. The adult moths became active at dusk and by a series of short irregular flights began a migration from the base of the trees toward the upper branches. Adults were found primarily on the lower leaves between 8 and 10 PM. By midnight they were spread throughout the tree, although a greater proportion appeared to be in the upper-half of the tree.

Adults were observed feeding on bits of aphid excrement on leaves. They also derive nourishment from the leaf teeth glands and particularly the petiolar glands. Reinke (1876 in Esau 1953) states that in Prunus the leaf teeth glands secrete resins whereas the petiolar glands produce a nectar.

At daybreak the mating behavior began, as the adults began to swarm in great numbers. Several hundred adults from the mating swarm were sexed, and all were males. Of 43 adults resting on cherry trees, 36 were females.

The females must emit a sex attractant, since many males simultaneously attempted to mate with a single female. Eventually 1 pair succeeded to copulate and continued to mate for 2 to 3 hr. Mating usually occurred on leaves, although any substrate sufficed. When mating was completed the pair parted and the male flew away. The female generally departed after about 30 sec without ovipositing. Within 3 hr after daybreak the adults had returned to the bark at the base of the trees and remained relatively inactive for the remainder of the day.

The number of times an individual mates is not known. The dissection of a female indicated a pouch laterally attached to the oviduct that appeared to be a spermatheca. Therefore, a single 2 to 3 hr copulation period should yield sufficient sperm to fertilize all of the female's eggs.

The entire mating procedure was witnessed in a leaf cage. With the pair facing in opposite directions, the male raised its wings vertically and joined its posterior to that of the female. The abdomens were not joined mesially. From a ventral view, the male was joined at the left of center on the female abdomen. The male then lowered its wings and the pair copulated for 2 hr and 19 min. Later examination revealed that females had asymmetrical genetalia. One opening, the ovipositor, was located in the center of the posterior. Another opening, the one used in copulation, was located laterad of the ovipositor.

Many unsuccessful attempts were made to witness the act of oviposition in the orchard. An adult resting on the underside of a leaf was located with the aid of a two-cell flashlight, then observed under illumination supplied by a one-cell penlight. The adult was collected with an aspirator when it started to move to another location. If it was a female, the possible oviposition site was marked on the leaf. These leaves were later examined in the laboratory but none showed any evidence of oviposition.

Although oviposition was not observed, it apparently takes place at night. Evidently the eggs are evenly distributed throughout the tree crowns, for mining appeared to be uniform throughout the crowns later.

The behavior of the adults at night may be a guard against desiccation. Adults under low humidity conditions in the laboratory died very quickly whereas those under humid conditions lived for longer periods.

Seasonal Development

To determine the rate of oviposition and to determine egg and larval development under field conditions, 50 to 100 leaves were selected at random to a height of 7 ft at various intervals from the Shelby and Lawrence orchards. The number of eggs, larvae and vacated mines from these leaves were recorded.

Estimates of actual field development of *N. slingerlandella* are given in Fig 9 and 10. The count on each date for each of the factors (emergence, hatching, etc.) reported was converted to a percentage of the total data collected for that factor. The arc sines of the percentages were graphed on their corresponding dates, and a line was fitted by eye to best represent the data.

As shown by the graphs, the time from mean emergence to mean oviposition was approximately 6.5 days at Lawrence and 10.5 days at Shelby. The time from mean oviposition to mean hatching was approximately 16 days at Lawrence and 19.5 days at

Fig 9.-Seasonal development of *Nepticula slingerlandella* in a tart cherry orchard. Lawrence, Michigan. 1967.

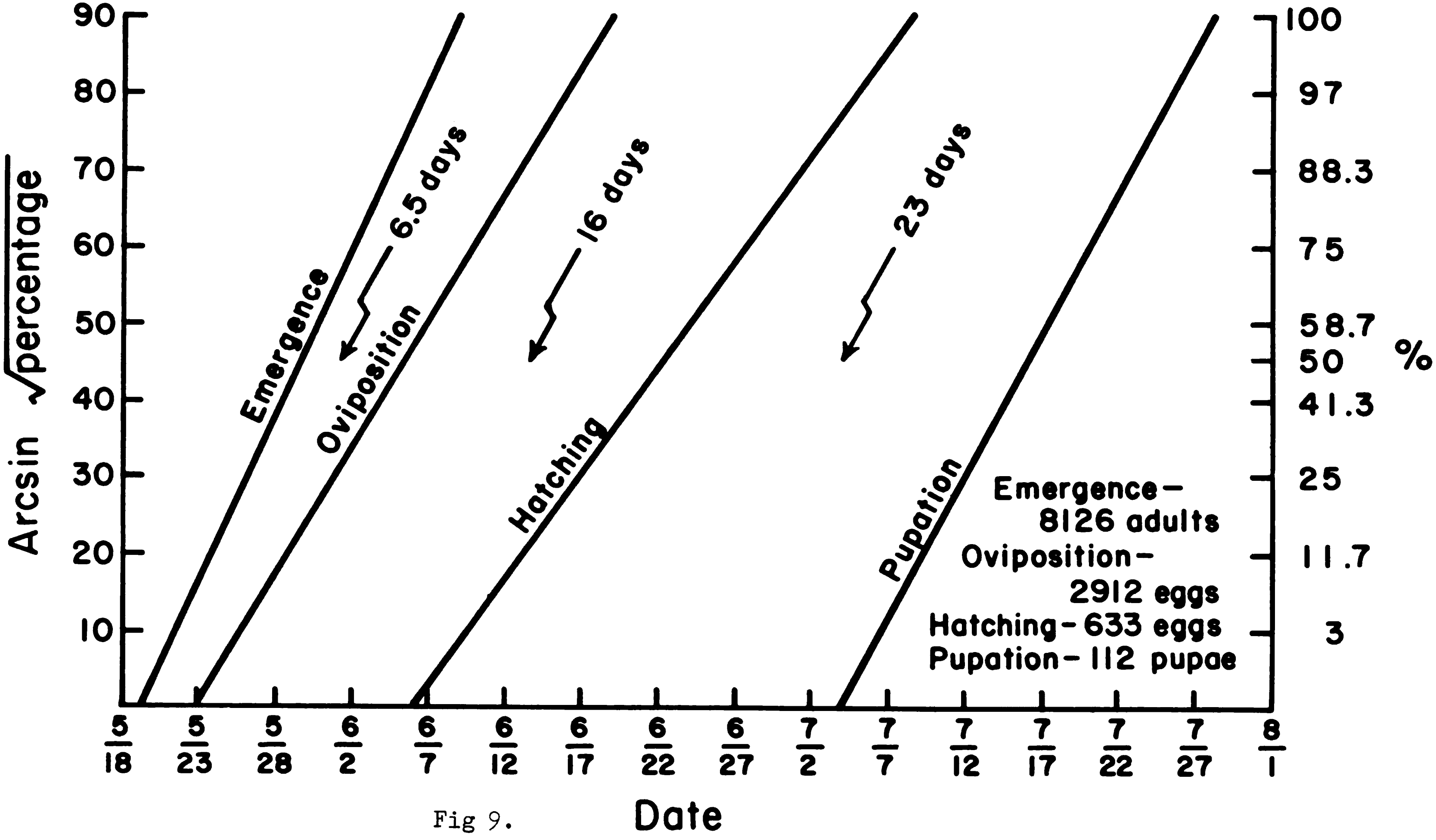
Arcsin √percentage
90
80
70
60
50
40
30
20
10
100
97
88.3
75
58.7
50
41.3
25
11.7
3
%
Emergence
Oviposition
Hatching
Pupation
6.5 days
16 days
23 days
Emergence-
8126 adults
Oviposition-
2912 eggs
Hatching-633 eggs
Pupation-112 pupae
5/18
5/23
5/28
6/2
6/7
6/12
6/17
6/22
6/27
7/2
7/7
7/12
7/17
7/22
7/27
8/1
Date

Fig 9.

Fig 10.-Seasonal development of *Nepticula slingerlandella* in a tart cherry orchard. Shelby, Michigan. 1967.

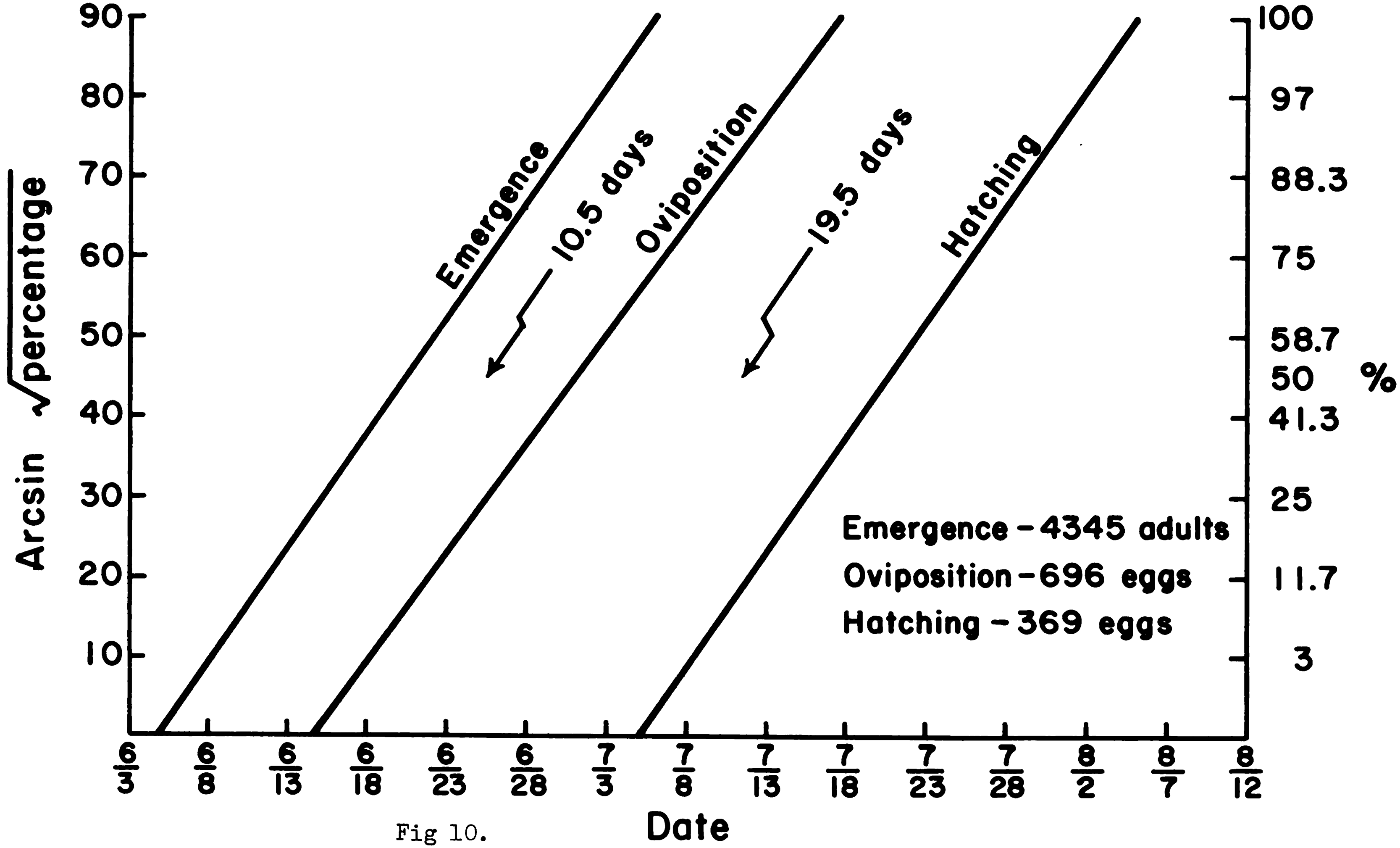
Arcsin √percentage
90
80
70
60
50
40
30
20
10
100
97
88.3
75
58.7
50
41.3
25
11.7
3
%
Emergence
10.5 days
Oviposition
19.5 days
Hatching
Emergence - 4345 adults
Oviposition - 696 eggs
Hatching - 369 eggs
6/3
6/8
6/13
6/18
6/23
6/28
7/3
7/8
7/13
7/18
7/23
7/28
8/2
8/7
8/12
Date

Fig 10.

Shelby. Mean larval development at Lawrence was completed in 23 days.

Longevity, Oviposition and Fecundity Studies

Leaf cages and branch sleeve cages were used in experiments in the Lawrence block in 1967 to determine longevity, oviposition potential, ovipositional rate, and mating behavior of *N. slingerlandella* adults. Eighteen individual leaf cages (Fig 11) were fabricated from 3/4 inch "Tygon" plastic tubing cut into 1 1/2-inch lengths. A bent pin-curl clamp was glued onto the side of each piece of tubing. A piece of thin, clear plastic, 1 inch sq. was glued on to the free end of the clamp so that it covered one end of the piece of tubing. A plastic foam gasket was glued onto the tubing underneath the plastic square. A 1-inch sq. piece of 32-mesh saran screen was glued to the other end of the tubing to allow ventilation. Two holes were made through the wall of the tubing with a paper punch. A small cork was placed in one hole, a novacaine vial (one end of which contained a small cotton plug) was placed in the other. The vial was filled with either distilled water or a saturated sucrose solution and was closed with a small red rubber stopper. The cage was then clamped onto a cherry leaf with the open end against the underside of the leaf.

For the first series of experiments, each vial was filled with distilled water, and a small sugar cube was added to each cage for nourishment. One male and one female adult

Fig 11-"Tygon" plastic leaf cage used in fecundity studies.

was placed in each cage. The cages were checked daily during the first 2 weeks of operation, followed by a period of 9 days during which no observations were made. The cage was removed when the female expired or escaped, and the date of death was recorded. The leaf was removed from the tree and the number of eggs on the underside was recorded at a later time in the laboratory.

A hard rain that occurred 10 days after the cages were placed in the orchard made the sugar cubes very sticky. Many weakened adults became entrapped in this sugar and perished prematurely. The substitution of vials filled with a sucrose solution eliminated this problem.

With prolonged use the tygon plastic became cloudy due to absorbed moisture and observation of adults in the cages became difficult. A few days in a dry environment made the plastic clear once again.

Eight females and 13 males died in the leaf cages from natural causes. The males and females lived an average of 9.4 and 9.6 days, respectively. The range of female longevity was 6 to 13 days, while male longevity ranged from 6 to 16 days.

Fecundity, as determined with individual leaf cages, is presented in Table 10. Variation in egg laying ranged from no eggs (10 females) to 82 eggs (1 female). Eight females laid at least 20 eggs. The mean fecundity was 11.4 eggs per female.

The average fecundity as established by the leaf cages is probably lower than that under natural conditions since the

Table 8.-Fecundity of Nepticula slingerlandella female moths in leaf cages on tart cherry trees. Lawrence, Michigan. 1967.

Observations	No. of eggs laid per female
1	82
1	47
1	41
1	29
2	27
2	20
1	19
1	11
3	10
2	9
1	8
2	7
1	6
1	3
2	2
4	1
10	0

insect was limited in its mobility and ovipositional sites. Transferring the cage to a fresh leaf daily would increase the number of available ovipositional sites, possibly increase the rate of oviposition, and probably increase the number of eggs laid per female.

To determine the fecundity under more natural conditions, 25 branch sleeve cages were placed in the Lawrence orchard. These cylindrical cages, measuring 8 inches in diam and 12 inches in length, were constructed of 32-mesh saran screen. Cloth tubes 8 inches in diameter were sewn on to each end of the screen to enable the ends of a cage to be tied around a branch. A 4-dram vial was filled with concentrated sugar solution, stoppered with cotton, inverted and fastened to the inside of the sleeve with a wire harness.

A week before adult emergence occurred five sleeves were placed on each of five trees selected at random in the Lawrence orchard; the cages were evenly spaced about 4 ft above ground level around each tree. Male and female adults were introduced into 20 cages and allowed to oviposit. Five cages (one per tree) served as checks. Most of the adults were collected from ground cages in the orchard to insure that none had previously oviposited. Approximately 1/4 of the sleeves were stocked with adults collected from the base of trees early in the adult emergence period.

Oviposition in the field was assumed to be completed when adults could no longer be collected. At that time the sleeves were collected and the eggs counted (Table 9). The mean fecundity was 17.9 eggs/female with a standard deviation of 7.4. An approximately normal fecundity frequency distribution is indicated in Table 10. One sleeve cage averaged 45 eggs per female. The factors responsible for this high count are not known.

Fecundity as established by the sleeve cages also may be lower than the actual field fecundity. The fact that some females were capable of laying 47 and 82 eggs, as demonstrated in the "Tygon" leaf cages, indicates that their mean reproductive potential may be much higher than 17.9 eggs per female. The average life span in the cages is not known. However, some adults were alive after 10 days.

Rate of Oviposition

Infested orchard litter, collected 2 weeks before adult emergence, was stored at 40 F prior to being placed in saran screen cages in the 70 F rearing room to initiate adult emergence. Unfortunately the prolonged storage period at cold temperatures, after the pupae had started the spring maturation process, reduced their viability so that only five female and eight male adults emerged. A pair of adults was placed in each of five "Tygon" plastic leaf cages (Fig 11) in an abandoned tart cherry orchard near East Lansing. The cages were moved to a fresh leaf each day and oviposition data recorded.

Table 9.-Fecundity of <u>Nepticula</u> <u>slingerlandella</u> female moths in branch sleeve cages on tart cherry trees. Lawrence, Michigan. 1967.

Females	Males (approximately)	Total eggs	Eggs/♀
10	25	450	45.0
10	17	270	27.0
10	12	242	24.2
10	13	238	23.8
11	15	221	20.0
10	20	200	20.0
12	16	239	19.9
11	17	203	18.5
10	15	184	18.4
9	15	166	18.4
10	15	165	16.8
10	20	167	16.7
9	15	132	14.7
12	15	162	13.5
11	15	144	13.1
10	20	127	12.7
10	16	124	12.5
10	12	122	12.2
10	20	110	11.0
15	15	93	6.2
210		3767	17.9

Table 10.-The fecundity frequency distribution of 20 branch sleeve cages on tart cherry that contained recorded numbers of *N. slingerlandella* adults. Lawrence, Michigan. 1967.

Class interval[a] (mean eggs/female)	Number of branch sleeve cages contained in the class interval
6-10	1
11-15	7
16-20	8
21-25	2
26-30	1
31-35	0
36-40	0
41-45	1

[a]Determined from a formula by Yule in LeClerg (1962). No. of classes = $2.5\sqrt[4]{\text{number in sample}}$.

Only two females oviposited. One female laid eight eggs, four each on her third and fourth days in the cage. The other female laid only two eggs (on her fourth day). The first female lived 10 days, the second, 8 days.

While the oviposition rate experiment was not entirely successful, coupled with the seedling tree experiment, it demonstrated that oviposition is possible within 2 days after emergence and usually is not completed in 1 day. Oviposition probably continues at a more or less constant daily rate until the female dies. In rare cases, field-collected leaves contained as many as a dozen eggs all deposited in a contiguous row, obviously extruded by one female. Usually eggs were deposited individually near a prominent leaf vein.

Egg Incubation Studies in the Laboratory

To determine the hatching time under constant temperature, 10 eggs obtained from the ovipositional rate experiment were kept at 80 F until hatching or desiccation occurred. Leaves containing the eggs were placed in a small jar that contained nutrient solution. The jar was placed in a 4-inch flower pot and a clear plastic freezer dish was inverted over it. This material was placed in an 80 F rearing room maintained at a 24-hour photoperiod which was supplied by three 40-watt fluorescent tubes. The eggs were examined daily. Of the 10 eggs, four failed to hatch, two hatched on the tenth day, three hatched on the eleventh day, and one hatched on the twelfth day. Mean time for egg hatching was about 11 days.

Larval Description

The first three larval instars are clear, glistening and semi-transparent. The last instar is translucent greenish white (Fig 12). The head capsule, barely visible in the first instar, takes on a brown coloration as the larva matures. It is extremely flattened with a somewhat triangular front and has two dark dorsal longitudinal lines which meld into an anterior triangle. From a lateral view, two dark thin lines meet anteriorly in a V shape. A large dark oscellus is present on each side of the anterior third of the head capsule.

The larva has three thoracic and nine abdominal segments. Segmented thoracic legs and crochets are absent. Paired locomotor protuberances are located on the venter of the second and third thoracic segments and on abdominal segments one through seven. The protuberances are apparent on a live specimen that is in the act of constructing its cocoon. They are extremely hard to detect on most preserved specimens. Each segment has two rounded lateral protuberances, one on each side, which are covered with fine light hairs. The body is covered with rather long, light colored, thinly dispersed, lateral and dorsal setae. At hatching, the first instar averages 0.09 mm in width by 0.46 mm in length. The last instar averages 0.36 mm in width and 4 to 5 mm in length.

Larval Instars

To determine the number of larval instars and the length of stadia, the width of larval head capsules was determined and

Fig 12.-Fourth instar N. slingerlandella larvae.

Fig 13.-Head capsule measurements of *Nepticula slingerlandella* larvae. Lawrence, Michigan. 1966-67.

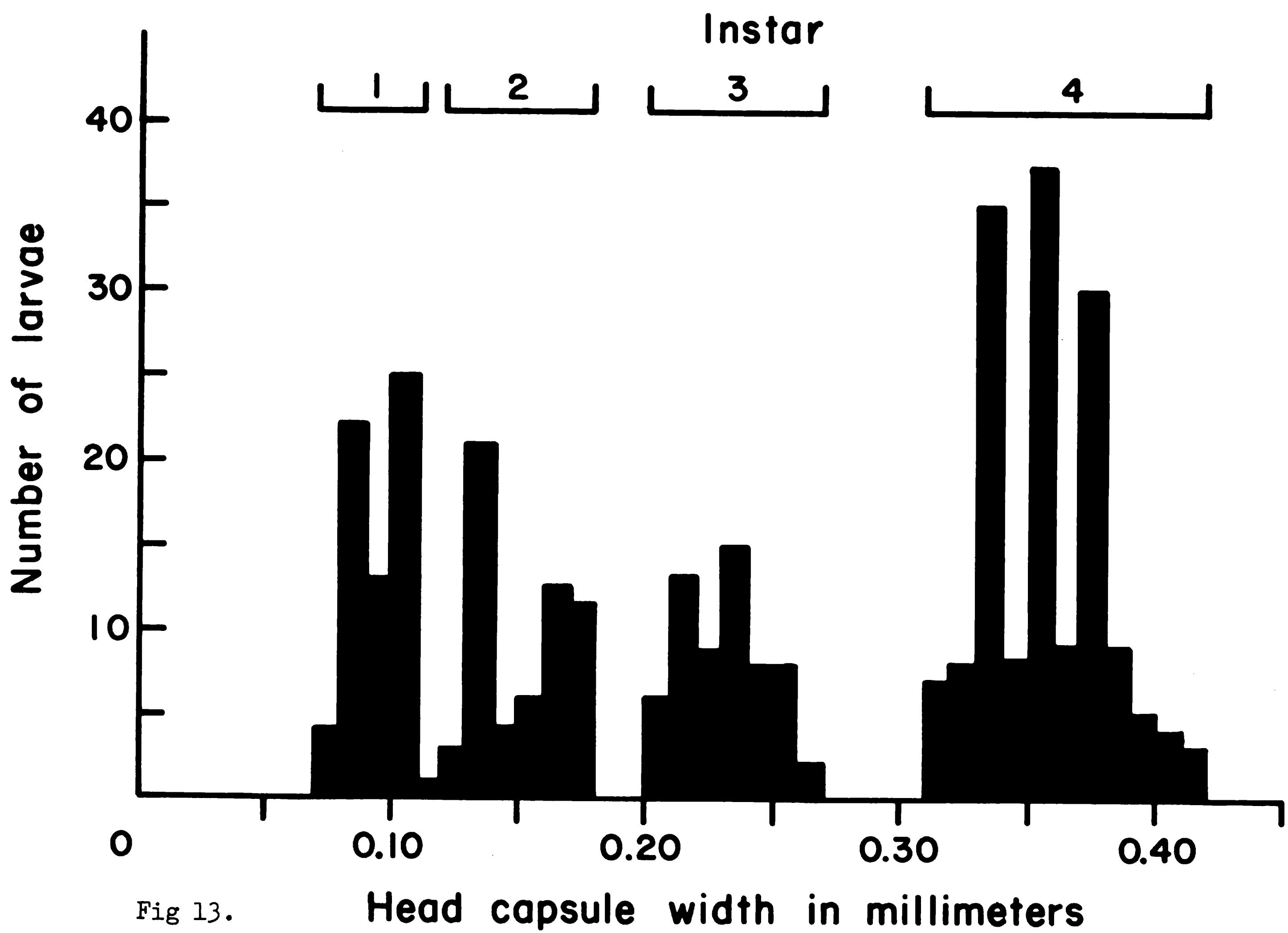
Instar
1
2
3
4
40
30
20
10
0
0.10
0.20
0.30
0.40
Number of larvae
Head capsule width in millimeters

Fig 13.

their date of collection noted.

The head capsule widths are reported in Figure 13. Instars one, three, and four are readily apparent. Head capsule widths for the first-instar ranged from 0.07-0.11 mm, for the third-, 0.20-0.26 mm and for the fourth-, 0.31-0.42 mm. The second-instar (0.12-0.18 mm), however, has a low count at 0.14 mm which makes it appear as two instars. It is the opinion of the writer that this low count is a coincidence. As a larva matured, the body width increased gradually while the head capsule width remained constant between molts. A larva that was ready to molt had a relatively small head capsule while the body had increased greatly in width. This caused the small head capsule to be pushed forward and gave the larva a very pointed anterior. This phenomenon was noted in each instar, but was never noted between 0.13 mm and 0.16 mm. In view of this, the writer determined that the larval head capsule widths between 0.12 and 0.18 mm represented only the second instar.

Nine head capsule exuvia, corresponding in width to those limits set for the third instar, were found on the bodies of recently molted fourth instar larvae. This verifies that these two instars are consecutive.

Larval Development

As indicated by the Lawrence seasonal development graph (Fig 9) the mean larval development is completed in 23 days.

Since the larvae have four instars, four stadia each of about 6 days are possible. This supposition is well supported if only the initial appearance of an instar is considered (Table 11). At least 6 days elapsed between the appearance of the first-and second-, and second- and third-instar. The third and fourth instars appeared simultaneously on July 2. Eighteen days had elapsed since the first-instar, hence, the fourth instar fits well. Some third-instar larvae should have been present on June 27 since more than 12 days had elapsed since the initial appearance of the first-instar.

If the data for each instar are subjected to an arcsine transformation and graphed, the results are as depicted in Figure 14. The first stadium is 11 days in length, the second, 2.5, the third, 5.5 and the fourth, 7 days. While the last two stadia concur closely with the field observations, the first two do not. If the second stadium was as short as indicated, then the leaves examined in the laboratory should have contained second-instar larvae for a shorter time than shown in Table 11. Further study is needed to determine the stadia length accurately.

Larval Parasitism

An incidence of larval parasitism occurred at Lawrence on August 10, 1966 when two small black pupae were found in two separate leaf mines occupied by desiccated *N. slingerlandella* larvae. The pupae were identified as members of the superfamily Chalcidoidea.

Table 11.-Larval instars of *Nepticula slingerlandella* and corresponding collection dates. Lawrence, Michigan. 1967.

Instar No.	Head capsule range (mm)	Date and no. of larvae								
		6/12	6/14	6/19	6/23	6/27	7/2	7/5	7/12	7/18
1	0.07-0.11	--	15	13	19	8	9	2	4	1
2	0.12-0.18	--	--	--	12	6	9	15	14	7
3	0.20-0.26	--	--	--	--	--	7	2	9	3
4	0.31-0.42	--	--	--	--	--	2	8	3	17

Fig 14.-Larval stadia of *Nepticula slingerlandella* as determined from larvae collected at Lawrence, Michigan. 1967.

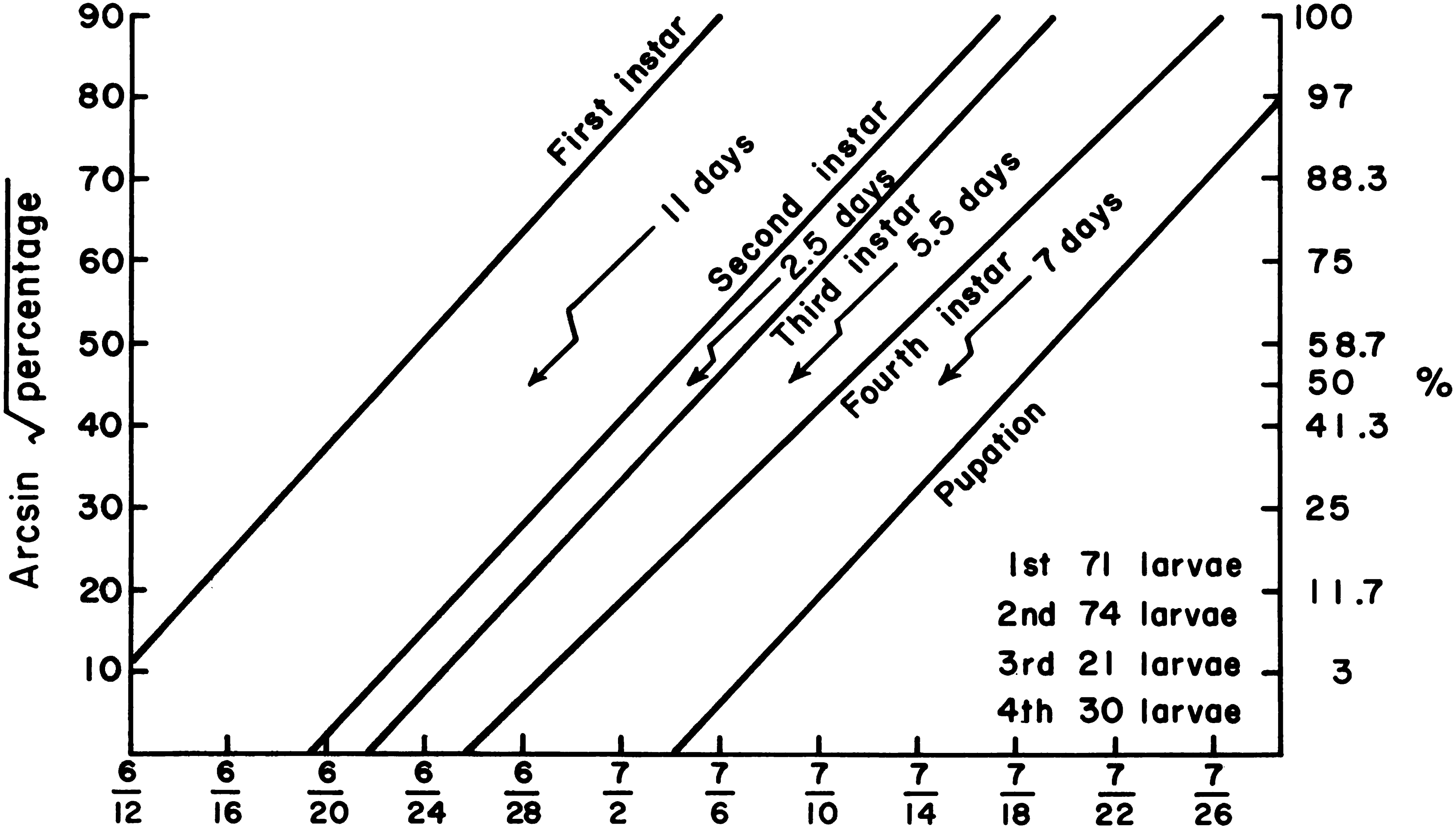
Arcsin √percentage
90
80
70
60
50
40
30
20
10
100
97
88.3
75
58.7
50
41.3
25
11.7
3
%
First instar
11 days
Second instar
2.5 days
Third instar
5.5 days
Fourth instar
7 days
Pupation
1st 71 larvae
2nd 74 larvae
3rd 21 larvae
4th 30 larvae
6/12
6/16
6/20
6/24
6/28
7/2
7/6
7/10
7/14
7/18
7/22
7/26
Date

Fig 14.

Approximately 50 expired larvae collected from Lawrence and Shelby were dissected from leaves and placed in humid containers at 80 F in an unsuccessful attempt to rear parasites. The dead larvae from Lawrence often exhibited a characteristic reddish-purple color on some parts of their body; many also contained fungal mycelium, which may be an indication that microorganisms attacked the larval forms.

Egg and Larval Survival

To obtain an estimate of egg and larval survival in Lawrence, mined leaves were gathered in mid-July of 1967 at random to a height of 7 ft. Of 210 eggs examined, 180 hatched; of these, 116 larvae expired, 47 pupated, and 17 were still mining in the leaves. This indicates an egg viability of 85.7%. Some of the 17 live larvae would undoubtedly perish before pupation; therefore, the larval survival is approximately 30%.

Description of the Mine

Each instar produced a characteristic mine. The first instar's mine was very fine and winding; the second, less winding and slightly larger in the diameter. The third instar's mine was relatively straight, again of a larger diameter. The blotch part of the mine (Figs 15 and 16), formed by the last instar, was usually rather oblong with the frass laid down in an irregular zig-zag pattern. The actual shape of the blotch depended on the relative

Fig 15.-Typical mines of Nepticula slingerlandella on tart cherry.

Fig 16.-Sequence of host plant leaf damage caused by Nepticula slingerlandella. An unmined leaf in the upper right, mines and some chlorosis in lower right, more severe yellowing with resulting defoliation at left.

interveinal space between the primary leaf veins. The last instar could not mine through the larger veins and was, therefore, limited in the leaf area that it could mine. If the larva happened to locate in an area where the veins were close together, as was usually the case on tart cherry, the blotch was rather long and narrow. If the interveinal space was wide, which was more usual on plum, then the blotch was round or oval with the frass laid down in a central heap at the base. The larva in the blotch was visible through the upper cuticle of the leaf. Its head moved slowly from side to side, while the posterior portion of the abdomen remained relatively stationary.

Pupal Development

The contents of many pupal cases removed from orchard litter in the fall of 1966 and spring of 1967 were examined for development, viability, and parasitism. Development of larvae that had been allowed to pupate in sterile greenhouse soil in humid, clear plastic breeding boxes also was observed.

After it drops to the orchard floor, a larva immediately spins a small, light colored cocoon in the orchard litter. The larva becomes more compact and wing pads begin to form as anterior lateral projections which lengthen posteriorly and curve beneath the abdomen (Fig 17). Legs and antennae form and are neatly placed between the wing pads. A transverse, mesially interrupted, row of short spines forms dorsally on

Fig 17.-Sequence of Nepticula slingerlandella pupal development. Youngest to oldest, left to right.

Fig 18.-Ventral view of an overwintering Nepticula slingerlandella pupa with part of its case removed.

the posterior of abdominal segments one through six, accompanied by dorso-lateral projections of abdominal segments one through seven. The eyes become black, while the head becomes orange. The wings and visible venter become a grey-yellowish-red while the dorsum of the abdomen remains green. The antennae lenghten while their annulations darken. This transformation to the overwintering form (Fig 18) is completed within a period of 6 weeks.

Three weeks prior to adult emergence the overwintering pupa begins the spring maturation process. The wing pads darken at the mid-point while the light fascia become faintly visible. Simultaneously the legs and antennae become a darker grey. The wings darken in both directions from the mid-point and the fascia become more apparent. The front femors darken, the wings become a uniform dark grey, while the dorsum of the thorax takes on a grey coloration. The abdomen becomes greyish while the antennae and front legs become a dark grey. The pupa at this stage closely resembles an adult encased in a skin (Fig 17) and undoubtedly is very close to emergence.

To obtain an estimate of the threshold temperature necessary for pupal development, infested orchard litter gathered on May 3, 1967 was placed in three closed, clear plastic pint containers and was incubated at 60, 70, and 80 F. Except for May 10, the samples were checked daily for adult emergence.

One adult emerged in both the 70 and 80 F containers on May 10 or 11 and in the 60 F container on May 15.

The threshold (T) was estimated by the following formulae:

at 60 F, K = (No. of days) (60 - T)

at 70 F, K = (No. of days) (70 - T)

at 80 F, K = (No. of days) (80 - T)

Where K = degree-day constant.

The number of days to emergence at 70 and 80 F was between 7 and 8 days. The mean (7.5) was used as the most reliable estimate. At 60 F emergence took 12 days. Therefore:

at 60 F, K = (12) (60 - T)

at 70 F, K = (7.5) (70 - T)

at 80 F, K = (7.5) (80 - T)

Since all equations equal K, T was found by algebraic substitution. The 70 and 80 F equations were each in turn compared to the 60 F equation.

At 70 F

$$12\ T - 7.5\ T = 720 - 525$$

$$4.5\ T = 195$$

$$T = 43.3\ F$$

At 80 F

$$12\ T - 7.5\ T = 720 - 600$$

$$4.5\ T = 120$$

$$T = 26.6\ F$$

The actual threshold undoubtedly lies between these limits, the mean, 34.9 F (approximately 35 F) is the best estimate of the pupal developmental threshold as shown by these data.

Pupal Survival

During the 3 weeks before 1967 emergence occurred at Lawrence, 335 pupal cases were removed from infested litter; of these, 193 contained dead pupae while 127 contained pupal skins indicating that adult emergence had taken place previously. Fifteen cases contained viable pupae while six, contained small holes with dead pupae inside (Fig 19), which indicated parasitism as reported by Crosby (1911). This indicates a pupal survival of about 40% with less than 2% parasitism.

Six out of the seven live pupae collected in the litter at Lawrence on May 13, 1967 had begun spring maturation. This indicated that most pupae emerged as adults the year following their entrance into the soil. It is possible that a small proportion emerge every other year. While 7 pupae is a small number on which to base an assumption, these did represent the number of live pupae found in 142 pupal cases collected throughout the Lawrence block.

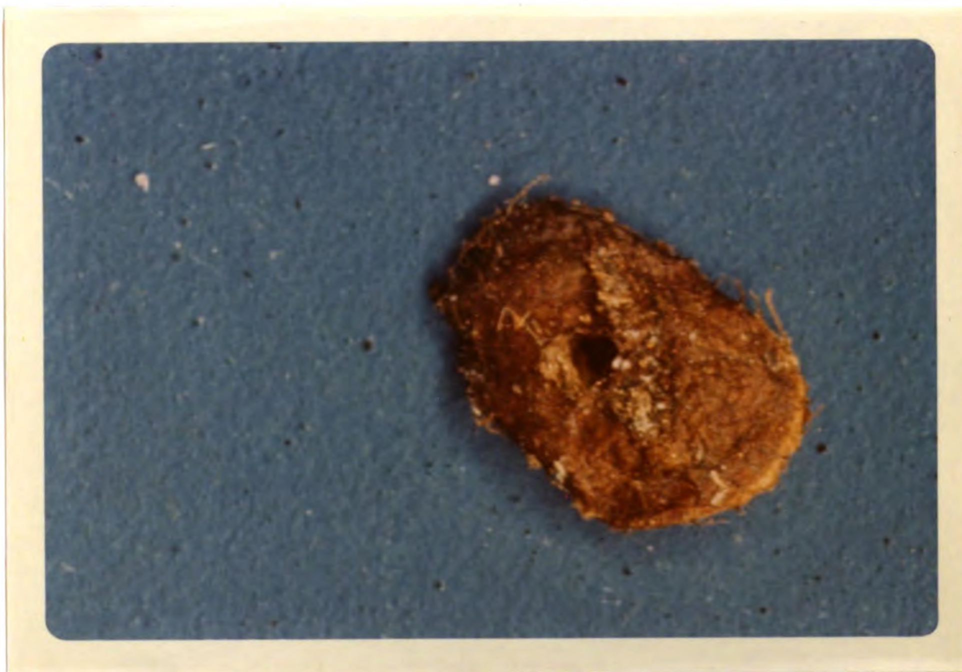

Fig 19.- Nepticula slingerlandella pupal case showing the emergence hole of a parasite.

Pupal Diapause

To determine if the pupae require a diapause, larvae from infested leaves were allowed to pupate in three closed clear plastic boxes held at room temperature. The boxes, which measured 8 x 12 inches were partially filled with moist, sterile greenhouse soil. At the end of 3 months no emergence had taken place and no pupae had matured past the overwintering stage.

Infested litter collected at Lawrence in November was placed in closed one-pint, plastic containers and refrigerated at either 25 or 40 F. One container was removed from each temperature cabinet for each of 4 weeks and incubated at room temperature. After 6 weeks at room temperature, adults emerged in the containers held at 25 F for 3 and 4 weeks. None emerged in the other containers. Thus a diapause appears necessary for this insect to complete its development.

Alternate Host Plant Studies

Sleeve cages were employed to test the response of *N. slingerlandella* to other possible host plants. Two sleeves were placed on pin cherry, *Prunus pennsylvanica* L., two on wild black cherry, *Prunus serotina* L., and three on peach, *Prunus persica* L. In addition, two seedling Stanley prune trees, *Prunus domestica* L., were placed in the Lawrence block. Additional field observations were made on wild black cherry trees located near Lawrence and Oshtemo, and on pin cherry trees in Lawrence and Bangor (Van Buren County).

There were no indications that N. slingerlandella attacked wild black cherry. An examination of leaves from the sleeves indicated that no oviposition had taken place.

Three second instar larvae were observed in two peach leaves removed from one sleeve. Other peach leaves indicated that limited oviposition had taken place. Evidently the larvae develop much more slowly in peach, since larvae in other hosts had pupated some time earlier.

The leaves of pin cherry were heavily attacked by N. slingerlandella and appeared to be as favorable a host as tart cherry. A suspected pin cherry infestation in Bangor where trees were heavily mined in 1966, was confirmed with the capture of one N. slingerlandella adult on a black sticky board.

The Stanley prune trees were very heavily attacked, with 88.5% of the leaves showing evidence of mining. Prune may be the favorite host of N. slingerlandella.

Meteorological Studies

The U.S. Weather Bureau publishes air temperature and growing degree day information useful in predicting certain natural phenomenon. The following experiments and observations were made to determine if these data could be used to predict N. slingerlandella adult emergence.

An experiment was initiated to ascertain the relationship between the temperature in the air and that which occurred simultaneously in the orchard litter, the resting place of the pupae. A thermistor was placed 1/4 inch below the litter surface 4 ft northeast of a tart cherry tree located at the Oshtemo orchard. The thermistor was connected by a 50-ft lead to an electronic unit which recorded the litter temperature continuously from May 3 through May 29, 1967. For an identical period, air temperature was recorded by a hygro-thermograph located at ground level 1 ft north of the thermistor.

A mean daily temperature was computed by averaging the temperatures recorded at 2 hr intervals by each instrument. The threshold temperature of the pupae, 35 F, was then subtracted and the remainder recorded as degree-days. The weekly and total summation is given in Table 12.

The litter registered about 150 more growing degree-days than did the air for identical periods and location. It appears, from this limited data, that air and litter temperature may not be sufficiently related to allow an accurate prediction of *N. slingerlandella* adult emergence from air temperature data.

To test this conclusion, air temperature data from Paw Paw, 10 mi east of Lawrence, and from Mears, 3 mi northeast of Shelby, was reviewed. When the pupal developmental threshold temperature (35 F) was used as the base, 680 spring growing degree-days were required for adult emergence at Lawrence in 1966 and 652 were required in 1967 indicating a close annual relationship. At Shelby comparable data were 1202 growing degree days in 1966 and 811, in 1967, demonstrating a poor annual relationship.

Table 12.-Air and orchard litter temperature summation from continuous temperature measurements. Oshtemo, Michigan. 1967.

Dates (inclusive)	Air Temperature		Litter Temperature	
	mean	degree-days 35 F	mean	degree-days 35 F
May 3 - 6	41	24	46	44
7 - 13	47	82	51	111
14 - 20	52	120	59	166
21 - 27	57	154	64	203
28 - 29	58.5	47	61	52
Total	50	427	56	576

Growing degree-days recorded after *N. slingerlandella* pupation in 1966 were added to the growing degree-days recorded until adult emergence in 1967. These totaled 3466 degree-days at Lawrence and 2918, at Shelby. It appears that air temperature summation will not consistently predict *N. slingerlandella* emergence.

Control Studies

In 1964, chemical control studies were conducted at the Lawrence orchard. A completely randomized block of 76 trees was used as the experimental design. Each chemical was replicated four times. Nine different insecticides, applied as single and double sprays, were tested for efficacy against adult moths. The chemicals were applied as a dilute spray to the point of runoff (about 10 gal per tree) with a hand held spray gun which operated at 400 psi.

On May 18, 1964 the chemicals were applied to all 72 test trees; four check trees were not sprayed; on June 1, 36 test trees received a second application. Each treatment was evaluated on July 9 for the presence or absence of mines through a check of 800 leaves (200 per tree) collected at random to a height of 7 ft (Table 13).

Azodrin (dimethyl phosphate, ester with *cis* 3-hydroxy-*N*-methylocrotonamide) gave outstanding results. Azinphosmethyl, RP 11974 (*S*-((6-chloro-2-oxo-3-benzoxazolinyl)methyl) *O,O*-diethyl phosphorodithioate) and GS 13005 (*S*-((2-methoxy-5-oxo-Δ^2-1,3,4, thiadiazolin-4-yl)methyl) *O,O*-dimethyl phosphorodithioate) also

Table 13.-The effect of various toxicants on Nepticula slingerlandella adults. Lawrence, Michigan. 1964.

Toxicant	pounds active material per 100 gal of water	% leaves infested Block 1	Block 2
Azodrin 3.2 EC	0.5	1	0
GS 13005 40 WP	0.5	8	3
RP 11974 4 EC	0.5	12	4
Azinphosmethyl 25 WP	0.25	3	6
GS 12968 40 WP	0.5	31	8
Mesural 50 WP	1.0	32	10
Diazinon 50 WP	1.0	47	11
Phosphamidon 4 EC	1.0	53	29
Check (average)	-	71	71

Block 1 treatments applied on May 18, 1964. Block 2 treatments applied on May 18 and June 1, 1964.

gave good control. Diazinon, GS 12968 (O,O-dimethyl-S-[5-ethoxy-1,3,4-thiadiazol-2(3H)-onyl-(3)-methyl]dithiophosphate) and Mesurol (4-(methylthio)3,5-xylyl methylcarbamate) gave adequate commercial control in the block that received two applications of material. Phosphamidon and carbaryl were not effective in either block.

In 1966, eight chemicals were applied at Lawrence as a single application to test their efficacy against first-instar larvae. A randomized block design which utilized 27 trees divided into three blocks of nine trees each was used as the experimental design. Each chemical was replicated three times. On June 25, each chemical was applied, using the 1964 method, to a separate tree selected at random in each block.

On July 15, each treatment was evaluated for the presence or absence of mines by checking 600 leaves (200 per tree) selected at random to a height of 7 ft (Table 14).

All insecticides tested gave good control. There were no significant differences (at 5% level) between chemicals, but all were significantly better than the check.

The formulas of those experimental chemicals that have not been previously given are: GC 6506 (dimethyl p-(methylthio) phenyl phosphate), NIA 10242 (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), Imidan (O,O-dimethyl S-phthalimidomethyl phosphorodithioate) and SD 8447 (2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethyl phosphate).

Table 14.-The effect of various toxicants on Nepticula slingerlandella eggs and larvae. Lawrence, Michigan. 1966.

Toxicant	pounds active material per 100 gals of water	% leaves infested[a]
GS 13005 40 WP	0.25	3.5
GC 6506 25 WP	0.25	4.0
RP 11974 30 WP	0.40	4.8
NIA 10242 50 WP	0.50	7.1
Azinphosmethyl 25 WP	0.25	9.9
Azodrin 5 EC	0.17	9.9
Imidan 50 WP	0.50	14.5
SD 8447 75 WP	1.50	15.8
Check	-	66.0

[a]No significant (5%) difference between chemicals; all were significantly better than the check at the 5% level.

In 1967, four chemicals were tested at Lawrence as ovicides, larvicides, or combination ovicide-larvicide treatments. A randomized block design, which utilized 39 trees divided into three blocks of 13 trees each, was used. Each treatment comprised a chemical, replicated three times, applied either as an ovicide (A), larvicide (B) or combination ovicide-larvicide (C). On June 8, A and C sprays were applied using the 1964 method. On June 19, B and C sprays were applied.

Each treatment was evaluated on July 12 for the presence or absence of mines by checking 600 leaves (200 per tree) randomly collected to a height of 7 ft (Table 15). In the larvicide treatments, only the blotch mines were counted. Many of the larvae were in their second-or third-instar when the sprays were applied and small thread-like mines were often visible.

The ovicide-larvicide sprays gave the most consistent excellent results. With the exception of NIA 10242, all insecticides performed well as ovicides; all, performed well as larvicides. All treatments were significantly better than the check. NIA 10242 was significantly less effective as an ovicide.

Nepticula slingerlandella may be effectively controlled in the egg, larva and adult stage with chemicals. Two sprays, spaced 10-14 days apart gave the most consistent control. A petal-fall spray followed by a first-cover spray against the adult is preferred. Second- and third-cover sprays against the

Table 15.-The effect of several insecticides on *Nepticula slingerlandella* eggs and larvae. Lawrence, Michigan. 1967.

Toxicant	pounds active material per 100 gals of water	% leaves infested[a]		
		A	B	C
Azodrin 3.2 E	0.50	0	1.2	0.16
GS 13005 40 E	0.45	3.8	1.7	0
Azinphosmethyl 25 WP	0.25	1.3	5.3	0
NIA 10242 50 WP	0.38	16.0	3.2	1.5

[a]The check trees had a mean infestation of 80.5%. A-ovicide, applied June 8, B-larvicide, applied June 19, C-ovicide-larvicide, applied June 8 and June 19. All treatments were significantly (5% level) better than the check. NIA 10242 was significantly less effective as an ovicide.

eggs and larvae also are effective, but some early mining and slight defoliation may occur.

Several organo-phosphorous, chlorinated hydrocarbon and carbamate insecticides gave effective control, therefore, cross resistance to insecticides should not be an important control problem for some time.

SUMMARY

Host plants of _Nepticula slingerlandella_ include tart cherry, sweet cherry, pin cherry and Stanley prune, _Prunus cerasus_ L., _P. avium_ L., _P. pennsylvanica_ L. and _P. domestica_ L. respectively. Limited mining occurred on peach, _P. persicae_ L., while wild black cherry, _P. serotina_ Ehrh., was not mined.

Adult emergence occurs once annually during full-bloom and petal-fall of tart cherry. Male emergence predominates during the first week. However, the sex ratio is 1:1 when peak emergence is reached 7 to 14 days later. Adult moths live an avg of 11 days.

Mating, preceded by swarming of the males, takes place shortly after daybreak and lasts for 2 to 3 hr. Oviposition occurs at night and usually begins within 2 days after mating. Eggs are evenly distributed throughout the tree on the underside of leaves often near a prominent vein. Mean oviposition climaxed 6.5 days after mean emergence at Lawrence and 10.5 days after mean emergence at Shelby. Fecundity, established by branch sleeve cages, averaged 17.9 eggs per female.

The larvae enter the underside of the leaves directly through the egg chorion. The period from oviposition to hatching averaged 17 days at Lawrence, 19 days at Shelby and 11 days at 80 F in the laboratory. The larvae mine the leaf palisade tissue predominantly, and are capable of causing premature

defoliation. Four larval instars were determined by head capsule measurements. Each instar produces a characteristic mine. The first-instar's mine is very narrow and winding; the second's, is less winding and slightly larger in diameter; the third's, is a relatively straight serpentine mine; and the fourth's, is a wide oval blotch which usually contains frass laid down in a zig-zag pattern. The larval stage averaged 23 days at Lawrence.

Pupation takes place in the orchard litter. In a matter of hours, a small, tan, oyster-like cocoon is constructed by the larva. Inside the cocoon the larva assumes pupal characteristics within a week and continues to mature for 5 weeks thereafter. It overwinters in this stage and the spring maturation process begins 3 weeks before adult emergence the following year.

Signs of very limited larval and pupal parasitism were found, but the parasites could not be identified. Attack of the larvae by microorganisms was suspected.

Chemical control studies indicated that insecticides applied in the petal-fall and first-cover sprays were effective in controlling the adult moths. Second- and third-cover sprays directed against the eggs and larvae were also effective, but some defoliation did occur.

LITERATURE CITED

Braun, A. F. 1917. Nepticulidae of North America. Trans. Am. Entomol. Soc. 43 : 155-209.

Crosby, C. R. 1911. The plum leaf miner. Cornell Univ. Bull. 308:218-228.

Crosby, C. R. 1912. Notes on the life history of Nepticula slingerlandella Kearfott (Tineidae). Can. Entomol. 44 : 25-27.

Esau, Katherine. 1953. Plant Anatomy. John Wiley and Sons, Inc., New York. 735 p.

Kearfott, W. D. 1908. North American tortricidae and tineina. J. New York Entomol. Soc. 16 : 187.

LeClerg, E. L., W. H. Leonard and A. G. Clark. 1962. Field plot technique. Burgess Publishing Company, Minneapolis, Minnesota. 373 p.

Lindquist, O. H. 1962. A biological study of a new leaf miner on birch, Nepticula lindquisti Freeman (Lep.-Nepticulidae) in Ontario. Can. Entomol. 94 : 525-530.

Needham, J. G., S. W. Frost and Beatrice H. Tothill. 1928. Leaf mining insects. The Williams and Wilkins Company, Baltimore. 351 p.

Slingerland, M. V. 1909. The plum leaf miner. New York Fruit Growers Proc. 8 : 122.